Structural and mechanistic relationships between nucleic acid polymerases

Rui Sousa

A superfamily of nucleic acid polymerases that includes the pol I and pol α classes of DNA-directed DNA polymerases, mitochondrial and phage DNA-directed RNA polymerases, and most RNA-directed polymerases may be defined on the basis of the occurrence of conserved sequence motifs and tertiary structure similarities between HIV-1 reverse transcriptase, DNA polymerase I and T7 RNA polymerase. Although sequence or structural similarities do not yet justify inclusion of the multi-subunit DNA-directed RNA polymerases in this superfamily, mechanistic similarities suggest a deep relationship between these and the simpler T7-like RNA polymerases.

THE TERTIARY STRUCTURE of a template-directed nucleic acid polymerase, that of the Klenow fragment of DNA polymerase I (DNAP I), was first described in 1985 (Ref. 1). Seven years were to pass before another polymerase structure appeared in the literature. During this period, development of the field depended largely on structure–function studies and on the identification of conserved sequence motifs among the increasing number of known polymerase sequences. These efforts culminated in an alignment that included most DNA-directed DNA polymerases (DNAPs), reverse transcriptases (RTs), RNA-directed RNA polymerases (RNAPs) and DNA-directed RNAPs (Ref. 2). However, the tenuous nature of many of these sequence similarities cast doubt on the entire scheme, which therefore awaited confirmation and refinement, or rejection, based on further structure–function or structural studies. The past few years have seen the emergence of the structures of three new polymerases[3–9], which now make it possible to evaluate the significance of these patterns of apparent sequence conservation.

Motif conservation

The pattern of polymerase motif conservation identified by Delarue *et al.*[2], Poch *et al.*[10] and Mendez *et al.*[11] can be seen in Fig. 1. Although more extensive patterns of sequence similarity within certain polymerase families have been identified, we focus here on the limited set of motifs that are most widely distributed. It can be seen that there is a correlation between polymerase template or substrate specificity and motif conservation. For example, motifs T/DxxGR and B are found only in polymerases that use DNA templates, while motifs B′ and D are restricted to polymerases that use RNA templates and, within the RNA-directed family of polymerases, motif E is restricted to polymerases that use dNTPs. Motifs A and C unify the RNA- and DNA-directed RNA or DNA polymerases because they occur in polymerases of either template or substrate specificity.

Relating sequence motifs to structure

It is instructive to examine the overall structures of these enzymes before looking at where the sequence motifs occur within them (Fig. 2). The similarity in the shape of the polymerase domains of T7 RNAP, p66 HIV-1 RT, and DNAP I to a 'cupped right hand' has led to the designation of the three subdomains of the polymerase domain as 'fingers', 'palm', and 'thumb'[4]. The most extensive similarity is seen between T7 RNAP and DNAP I : the folding and almost all of the secondary structure in their respective polymerase domains is nearly identical, while the structural similarity with HIV-1 RT is limited to a core comprising most of the palm subdomain.

Motifs A and C. Peering more deeply into the large template-binding clefts of these enzymes, we can localize active sites that have been defined by structure–function studies, structural studies of polymerase–substrate/template complexes, and sequence comparison (Fig. 3). Most of the residues forming these active sites are part of the sequence motifs shown in Fig. 1. Motifs A and C form three strands of a β-sheet and a short segment of α-helix within the core of the palm subdomain, which is structurally similar in RT, T7 RNAP and DNAP I. Two amino acids (Asp537/Asp812 in T7 RNAP; Asp705/Asp882 in DNAP I; Asp110/Asp185 in HIV-1 RT), which are identified as invariant within these motifs, are brought into alignment when the three polymerases are superimposed. These two Asp residues bind and present two metal ions in the appropriate geometrical arrangement to catalyse a phosphoryl transfer reaction at the active site[12]. A third well-conserved carboxylate (Glu883 in DNAP I; Asp186 in RT; absent in T7 RNAP) is also expected to be involved in catalytic metal binding. Significantly, mutation of this third carboxylate reveals that it is less critical for activity than either of the two invariant Asp residues[13,14].

Motifs B and B′ are located in the fingers subdomains of DNAP I, T7 RNAP and RT, respectively. While motifs B and B′ are dissimilar in sequence and structure, and occur within a subdomain that is structurally dissimilar in the RNA-directed versus DNA-directed polymerases, they are similarly positioned relative to the center of the active site in both classes of polymerases. In the structure of HIV-1 RT complexed with primer–template, the fingers subdomain and elements from motif B′ contact the template strand[5]. Modeling, structural and mutation studies imply that a region in the corresponding position (including elements of motif B) of the fingers subdomain of DNAP I or T7 RNAP would be similarly involved in contacts with the template strand[15]. As the template in the RT primer–template structure does not extend downstream of the 3′ end of the primer, downstream template contacts must be deduced from modeling. The more compelling model would place the downstream template contacts on β-strands 3 and 4 of RT, the loop between these strands and (perhaps) the carboxy-terminal region

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 0968–0004/96/$15.00 PII: S0968-0004(96)10023-2

of β-strand 11a (Ref. 16). However, it is also possible that these elements are involved in substrate contacts[17,18]. The latter hypothesis would be consistent with recent evidence from structural and mutational analyses that the amino-terminal region of motif B (helix O) in DNAP I interacts with the dNTP phosphates and ribose moiety[19,20]. The docked dNTP modeled by Arnold and colleagues in the RT primer–template complex would not contact the fingers subdomain, but would instead establish contact with elements of motifs A and C and (possibly) with β-strand 11 (Ref. 16). It is, therefore, unclear if the fingers subdomain is involved in substrate as well as template strand contacts, or if this represents a case where analogous structures in different polymerases have different functions (i.e. a role in substrate binding for the fingers of DNAP I and T7 RNAP, but not for RT).

Functional roles of the T/DxxGR motif and motif E. Irrespective of the question of substrate binding, it is clear that the fingers subdomains and elements of motifs B and B′ are involved in template-strand binding in both the DNA-directed and RNA-directed polymerases. It is, therefore, intriguing that structural similarity in the fingers subdomain and conservation of motifs B and B′ reflect polymerase template specificity. In the same way, we can examine the location and proposed function of the T/DxxGR motif, which occurs in the DNA-directed polymerases, but not in the RNA-directed polymerases. Mutational studies and modeling of template–DNAP I or template–T7 RNAP structures based on the RT primer–template complex reveal that the structure formed by this motif is also involved in template-strand contacts[15]. Along similar lines, it may be noted that motif E, the only one of five motifs conserved in RNA-directed DNA polymerases that is not also conserved in the RNA-directed RNA polymerases, forms a structure designated the 'primer grip', which is intimately associated with the primer strand[5].

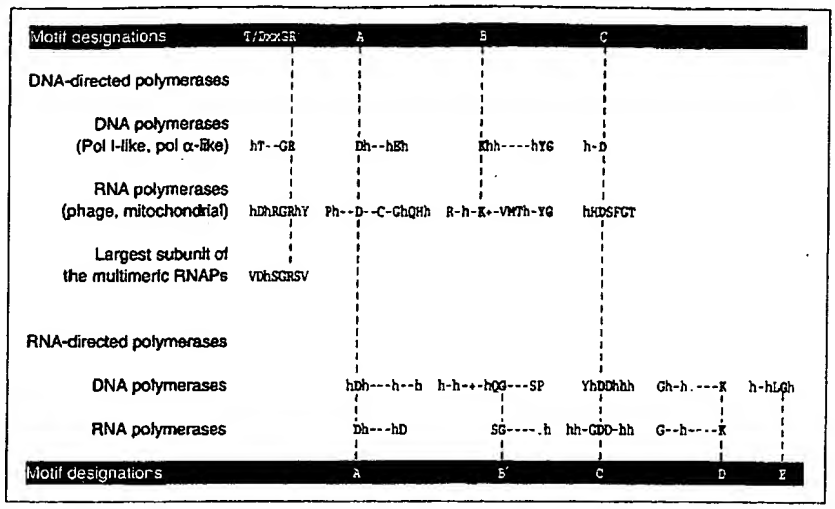

Figure 1

Patterns of motif conservation in nucleic acid polymerases[2,10,11]. Residues in blue are invariant. Other residues given are well conserved: h, hydrophobic residue; +, positively charged residue; −, any residue; ., a sequence gap. Residue numbers of invariant residues in DNA-directed DNA polymerase (DNAP) I, T7 DNA-directed RNA polymerase (RNAP) and HIV-1 reverse transcriptase (RT) are: for T/DxxGR motif – DNAP I, Arg668; T7 RNAP, Arg425; for Motif A – DNAP I, Asp705; T7 RNAP, Asp537; RT, Asp110; for Motif B – DNAP I, Lys758/Tyr766/Gly767; T7 RNAP, Lys631/Tyr639/Gly640; for Motif B′ – RT, Gly152; for Motif C – DNAP I, Asp882; T7 RNAP, Asp812; RT, Asp185; for Motif D – RT, Lys220; and for Motif E – RT, Gly231.

Structural differences between T7 RNAP and DNAP I. It is intriguing that there is one region where DNAP I and RT are more similar to each other than to T7 RNAP, even though DNAP I and T7 RNAP show greater structural similarity overall. RT and DNAP I both exhibit a fourth β-strand (β-strand 14 in DNAP I; 11a, 11b in RT), which extends the three-stranded sheet formed by motifs A and C. T7 RNAP lacks this fourth β-strand. As DNAP I and RT share substrate (and

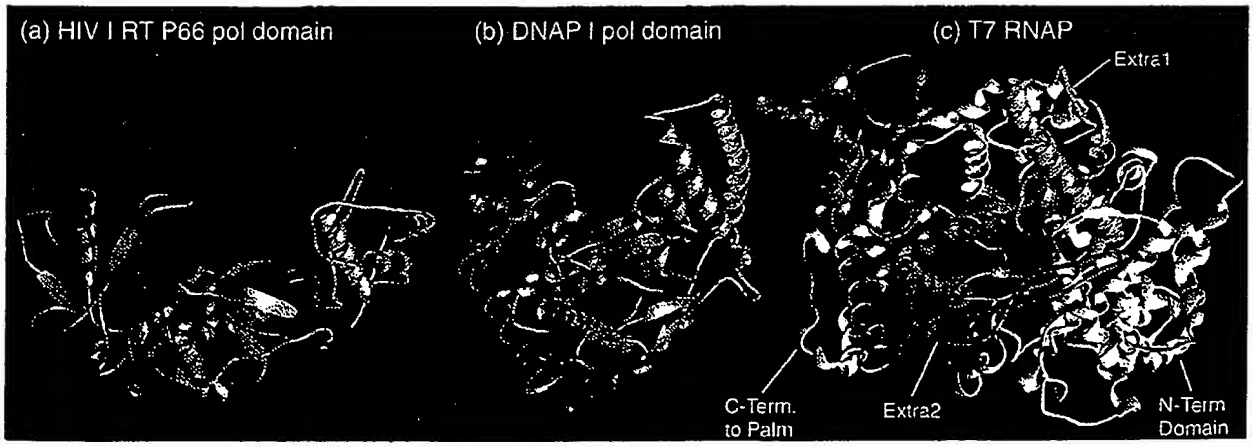

Figure 2

Structures of the polymerase domains of **(a)** P66 reverse transcriptase (RT), **(b)** DNA-directed DNA polymerase (DNAP) I and **(c)** the complete T7 DNA-directed RNA polymerase (RNAP) molecule[1,3–9]. The 'thumb' subdomains are colored green, the 'palm' subdomains are in red, and the 'fingers' subdomains are blue. Structural elements in T7 RNAP that have no counterpart in the DNAP I polymerase domain are colored light gray ('Extra1', 'N-Term' domain, 'C-Term to Palm') or orange ('Extra2'). The single magenta-colored helix in DNAP I and T7 RNAP is not formally considered part of the polymerase subdomain, but is conserved between T7 RNAP and DNAP I. The two green-colored spheres mark the positions of the invariant Asp residues, which identify the center of the active site.

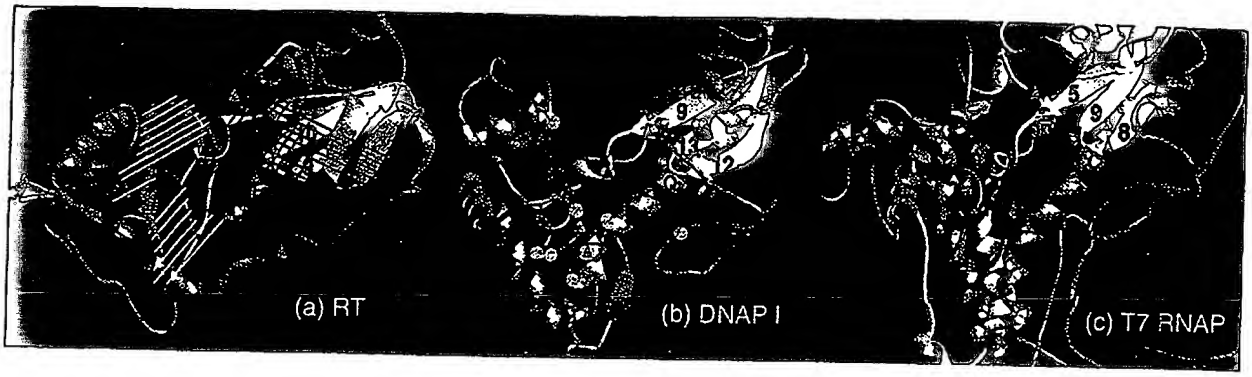

Figure 3

Polymerase domain structures of **(a)** reverse transcriptase (RT), **(b)** DNA-directed DNA polymerase (DNAP) I with the 'thumb' subdomains removed to allow an unobstructed view into the active site, and **(c)** the T7 DNA-directed RNA polymerase (RNAP) structure with the thumb and amino-terminal domains removed. The polymerases are presented with the long axis of the template-binding cleft parallel to the long axis of the page. RT helices E, F and strands 6, 9 and 10 correspond to DNAP I helices Q, R and strands 9, 12, 13, and to T7 RNAP helices CC, DD and strands 5, 8 and 9, respectively. The structures formed by motifs A and C are in yellow; motifs B and B′ are colored magenta; and the T/DxxGR motif (strands 3 and 4 in T7 RNAP; 7 and 8 in DNAP I) is orange. Motif E is colored green. Elements of the 'palm' not part of these motifs are in red, and the 'fingers' are blue. The carboxyl terminus of T7 RNAP is marked by an asterisk. T7 RNAP structures not a part of the polymerase domain are in light gray or orange. The region of RT in contact with the template strand in the RT–primer–template structure is indicated by diagonal white lines[5]. The diagonal yellow lines indicate where additional template-strand contacts might be made if the template strand were extended beyond the length in the crystal structure of the complex[16]. The region in contact with the primer strand is in indicated by diagonal blue lines[5]. Elements of the RT molecule that could contact a substrate dNTP in the RT active site are indicated by diagonal magenta lines[16]. Green triangles indicate the locations of mutations that affect NTP K_m* or nucleoside analog utilization, but whose effects are thought to be mediated by effects on template binding[21]. Magenta triangles indicate mutations that affect NTP K_m* or nucleoside analog utilization or become crosslinked to NTP, and whose effects are interpreted to be owing to direct NTP contacts[13–15,19–21,49,50]. Blue circles indicate the positions of mutations that affect K_d* for template or become crosslinked to the template and whose effects are interpreted to be owing to template-strand contacts[13–15,51]. Magenta circles indicate mutations that increase template K_d* and whose effects are interpreted to be owing to primer-strand contacts. The green spheres indicate the positions of the catalytic Asp residues. K_m* and K_d effects are fivefold or greater.

product) specificity, one possibility is that this structural divergence between the DNAPs and the RNAP is related to substrate/product specificity. Such a speculation is supported by the proposal that the AZT-resistance mutations at Lys219 of RT β-strand 11a exert their effects directly through contact with the dNTP (Ref. 21). It is also supported by the observation that mutations of Phe882 at the carboxyl terminus of T7 RNAP (which superimposes on Lys219 of RT) increase rNTP K_m (Ref. 22).

Alternatively, this structural pattern might be related to the fact that T7 RNAP uses a double-stranded template while DNAP I and RT use partially single-stranded templates. A fourth β-strand positioned in T7 RNAP analogously to the β-strand seen in DNAP I and RT could clash sterically with the domain we call 'Extra1', which is present in T7 RNAP, but not DNAP I or RT, and which might be involved in unwinding. This fourth β-strand could also occlude a groove in T7 RNAP in which the unwound non-template strand might bind. Truncation of the carboxyl terminus of T7 RNAP to remove the fourth β-strand might then reflect the need to remove structures that would sterically clash with the unwound non-template strand or with the protein domains responsible for unwinding.

Summary of motif structure–function correlations. Within a superfamily that includes the DNA pol I class of enzymes, the phage and mitochondrial RNAPs, the majority of RNA-directed polymerases, and (perhaps) the pol α class of enzymes, we can catalogue a set of correlations between conservation of structural elements and the functions of those elements. Motifs A and C, and most of the palm subdomain, are conserved irrespective of polymerase–template or substrate specificity, reflecting a direct role for these structures in the activity common to all polymerases: phosphodiester bond formation. Conservation of motifs T/DxxGR, B and the fingers domain in the DNA-directed polymerases, and conservation of motif B′ and a distinct fingers domain in the RNA-directed polymerases reflects a role for these elements in template-strand binding. The unique position of Motif E, as the motif that is conserved only in the DNA-synthesizing polymerases within the RNA-directed class of enzymes, reflects the role of this structural element in product (i.e. primer) contacts. Finally, structural similarity in β-strands 14 and 11 of DNAP I and RT, respectively, and divergence with T7 RNAP in the corresponding region might reflect a role for this structural element in substrate/product contacts or the structural requirements for utilization of a double-stranded template.

Modularity in polymerase architecture

One way to look at polymerase structure is to imagine that polymerases are assembled from modules whose structural conservation often reflects common function, as illustrated in Fig. 4. The fingers, palm and thumb subdomains together form the polymerase domain. The thumb subdomains, whose functions have not yet been addressed, are the least well conserved of these three subdomains, but appear to be similar in certain general features: they are extended, flexible, predominately α-helical structures that are involved in conferring processivity on polymerization by wrapping around bound template and/or interacting directly with the template to inhibit polymerase–template dissociation[23–26].

The function of the complete polymerase domain appears limited to the minimal function of template-directed processive polymerization. Specific polymerases can display additional activities, but these activities appear to reside on distinct domains. Thus, T7 RNAP is capable of sequence-specific (promoter) DNA binding and template unwinding. It emerges that promoter-specific binding may be largely conferred on T7 RNAP

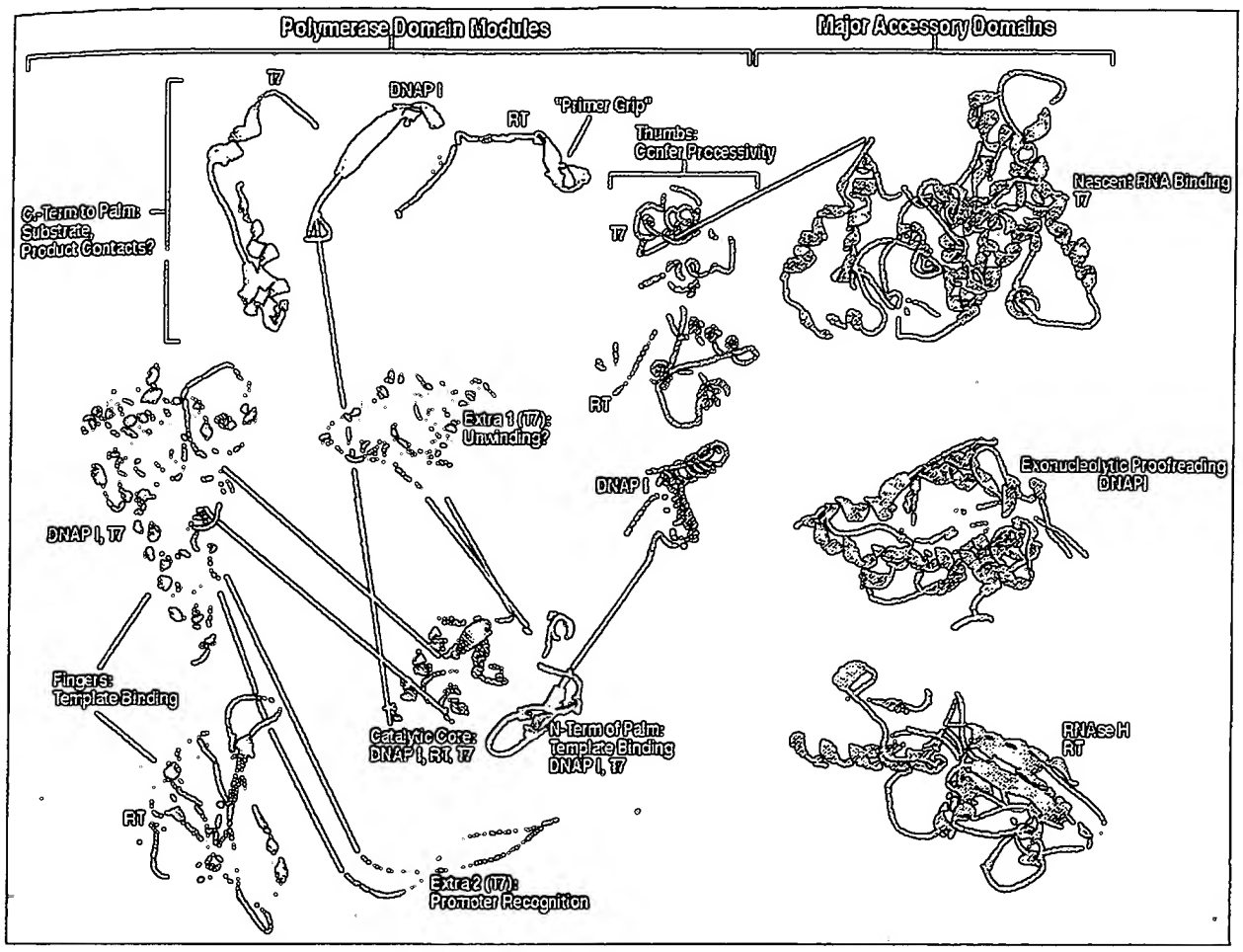

Figure 4

A modular architecture for polymerases is suggested. The white lines indicate how different structural elements are linked to each other. The functions of these structural elements and their occurrence in different polymerases are indicated.

by the 'Extra2' domain (Fig. 4), as amino acids critical for promoter recognition map to this loop[27,28]. Template-unwinding activity in T7 RNAP has not been mapped, but it is possible that the Extra2 domain plays a role in this process as it is placed at the leading end of the polymerase and forms two grooves into which the unwound strands of DNA might fit. The major accessory domains of each of these polymerases (RNAse H in RT; Exo proofreading in DNAP I; amino-terminal domain in T7 RNAP) are responsible for distinctive activities in each enzyme and bear no structural similarity to one another, although they do occupy roughly equivalent positions so that they can interact with nucleic acid strands upstream of the polymerase active site.

The scheme for polymerase organization presented in Fig. 4 suggests that gene fusion and recombination events might have played a role in the evolution of modern multidomain polymerases from multi-subunit enzymes composed of more simple polypeptides. In this light, the structure of the middle RNAP of phage N4 is intriguing because it shows homology to the complete T7 RNAP polymerase domain, but is composed of two subunits (designated P4 and P7; L. Rothman-Denes, pers. commun.). The break in the N4 RNAP places the amino terminal of the palm and thumb subdomains onto one subunit, and the conserved catalytic core and fingers structures on the other. Thus, in this instance, the subunit separation coincides with one of the subdomain divisions presented in Fig. 4.

Structure and extent of this polymerase superfamily

Using the alignments of Delarue *et al.*[2] and Poch *et al.*[10] as a guide, it is expected that the pol I class of DNAPs and the phage and mitochondrial RNAPs will display polymerase domain structures with palms and fingers similar to those shown in Fig. 4, while the structures of the thumb subdomains might show variation within the range revealed in this figure. The alignment by Delarue *et al.* included the pol β class of DNA polymerases in its scheme for unification, but also suggested that the pol β class was exceptionally divergent because it was unique among the DNA-directed polymerases in lacking an identifiable motif B sequence. Analysis of the X-ray structure of pol β suggests that, in fact, this polymerase is not related to DNAP I, RT or T7 RNAP and might be more closely related to nucleotidyl transferases, which are not polymerases[29]. The pol α class of DNA polymerases is expected to display a palm like that of DNAP I or T7 RNAP, however, changes in the spacing between motifs A, B and C imply at least some topological rearrangements in the fingers domain of the pol α class of polymerases relative to the pol I class. For the RNA-directed polymerases,

analysis of the spacing between motifs also suggests that the palm domain structures will be relatively invariant, while there might be variations in the fingers-domain structures of the most distantly related classes. Alternatively, variations in motif spacing could be accommodated in 'extra' modules and variation in lengths of different elements on the periphery of these domains, which would leave the folding of their cores unchanged (as is seen, for example, in the comparison of the T7 RNAP and DNAP I structures).

Status of the multi-subunit RNAPs

Not included in the Delarue *et al.* alignment were the multi-subunit RNAPs (prokaryotic RNAPs, eukaryotic RNAPs I, II and III). The identified sequence similarities between the catalytic subunits of these RNAPs and polymerases of the RT-DNAP I–T7 RNAP superfamily[11,30,31] are not extensive enough to be conclusive without confirmation from structures at atomic resolution and, despite observations of similarity in the overall shape of the multi-subunit RNAPs and T7 RNAP[32–34], it might be more profitable to compare the extensive mechanistic, rather than the structural, similarities of these enzymes. These include similarities in the timing, triggering and conformational changes involved in the transition from the poorly processive initiation phase of transcription to the elongation phase[35–41], and the utilization of similar promoter[42,43] and terminator sequences[44–47] by both classes of polymerases. The observation that the yeast RNAP holoenzyme is a dimer composed of a core enzyme that is homologous to T7 RNAP and a polypeptide that is functionally and structurally similar to the sigma subunit of *Escherichia coli* RNAP[48], might also be relevant to understanding the relationship between the single subunit and multi-subunit RNAPs.

Concluding remarks

The significance of the observed pattern of structural similarities between nucleic acid polymerases, the correlations between structural similarity and the functional role of different structural elements, and the mechanistic similarities between the multi-subunit RNAPs and simpler polymerases like T7 RNAP have yet to be fully explored. Do the observed patterns reflect strict functional requirements for the utilization of different templates or substrates, or do they reflect contingent events in the evolutionary history of polymerases? To what degree can the different functionalities displayed by a polymerase like T7 RNAP be distinctly mapped to different structural modules? Studies aimed at answering these questions and others might profit from a perspective that integrates the recognized structure–function relationships of nucleic acid polymerases.

Acknowledgements

Work in the author's laboratory is supported by NIH grant GM52522-01A1.

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PRINTED IN THE UK. ISSN: 0968-0004
JOURNAL NUMBER: 02115

May 1996 (245) Volume 21
No. 5 pp. 161–196 ISSN 0968-0004

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COVER

The Rho family of GTP-binding proteins play a role in the organization of the actin cytoskeleton. Recently, members of this family have also been shown to function in cell proliferation and in the activation of transcription through signaling cascades. On p.178 of this issue, Marc Symons discusses this bifurcation of function.

The cover shows a micrograph of a phalloidin-stained rat fibroblast expressing constitutively active Rac (a member of the Rho GTPase superfamily). With thanks to M. Symons for the micrograph.

Cover design by Nigel Hynes.